Open Access

J. Glob. Innov. Agric. Sci., 2024, 12(1):101-104
ISSN (Online): 2788-4546; ISSN (Print):2788-4538
DOI: https://doi.org/10.22194/JGIAS/12.1150
http://www.jgiass.com

Society for Innovative Agriculture

Polymorphism of the DGAT1 gene and its relationship to milk yield and some chemical properties of milk during the first stage of production in local cows and Holstein cows; a comparative study

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In present study, 50 dairy cows of the local and Holstein breeds were used (34 local cows and 16 Holsteins). Milk samples were collected from 50 cows once every two weeks from the morning milking (to estimate the daily amount of milk, multiply the amount by 2). One-day test to calculate the daily and monthly milk yield. Samples were taken at a rate of 50 ml for each cow, and the proportions of the main milk components (fat, protein, lactose and non-fat solids) were estimated. Blood samples were drawn from cows for the purpose of DNA extraction. Then the DGAT1 gene was amplified using the primers of the studied piece, which was 411 bp in size. The gene was cut using Taq1 restriction enzyme. The results showed the presence of three genotypes: AA, AK, and KK. In terms of the quantity of daily, weekly, monthly, and first stage milk production, the findings also demonstrated that the genotypes AK and KK of the Holstein breed were superior ($P< 0.05$) than the two genotypes AA and AK of the local breed. While the genotype AA of the local breed was superior to the genotype AK in terms of daily, weekly, and monthly milk production as well as the first stage, it was also discovered that there is a correlation between the genotypes and the chemical components of milk, as the results showed that the genotype AA was significantly superior ($P<0.05$) by the percentage of fat in the local breed on the AK and KK genotypes of both breeds.

Keywords: DGAT1 gene, genotypes, milk production, fat percentage, Holstein cows, Protein, Lactose, Non-fat solids, DNA extraction.

INTRODUCTION

Milk fat is a triglyceride consisting of (one part of glycerol and three fatty acids), which is one of the most important indicators of milk quality (Li *et al*., 2021). The nutritional value of milk and dairy products is closely related to the fat content in milk, as it constitutes approximately 30% of the total fat consumed in the human diet. Milk fat consists of a complex mixture of fats, which consists of 70% of saturated fatty acids and 30% of polyunsaturated fatty acids (Rajesh *et al*., 2022). Diacylglycerol acyl-COA acyltransferase (DGAT1) gene is a promising candidate gene for milk production traits due to its important role as a key enzyme in catalyzing the final step of triglyceride synthesis. Thus, the bovine DGAT1 gene can be used as a marker for milk production in cattle (Samuel *et al*., 2022). Which is a protein that catalyzes the last step in the synthesis of triglycerides, as it encodes the enzyme Acyl-COA-diacylglycerol acyltrans ferase, which plays an important role in the synthesis of diglycerols in physiological processes in the cell such as the formation of adipose tissue, the absorption of fats through the intestine, and the assembly of lipoproteins (Binding fat with protein). The activity of the (DGAT1) gene was described for the first time by Weiss and Kenned (1956) in the fifties of the last century, as this gene is considered one of the first genes that were identified. It is located on the central end of the fourteenth bovine chromosome. The gene consists of 14,117 bp. The DGAT1 gene was a functional candidate gene for milk production traits (Mohammed *et al*., 2015). Agrawal *et al*. (2018) found that the study on Sahiwal cows in India demonstrated that the quantitative trait locus (QTL) has a significant impact on the composition and production of milk, DGAT1 is directly responsible for an estimated 50% variation of the milk's fat content as well as the amount of milk produced in dairy cow.

ALajwadi, I.M.N. and J.M. Owaid. 2024. Polymorphism of the DGAT1 gene and its relationship to milk yield and some chemical properties of milk during the first stage of production in local cows and Holstein cows; a comparative study. Journal of Global Innovations in Agricultural Sciences. 12:101-104.
[Received 5 Jul 2023; Accepted 28 Aug 2023; Published 16 Mar 2024]

MATERIALS AND METHODS

***Milk components analysis*:** Milk samples were collected from 50 cows once every two weeks from the morning milking (to estimate the daily amount of milk, multiply the amount by 2). One-day test to calculate the daily and monthly milk yield. Samples were taken at a rate of 50 ml for each cow, and the proportions of the main milk components (fat, protein, lactose and non-fat solids) were estimated using the device used for this purpose, the German-made Gerber Funke Lacto Flash.

***Determine DGAT1 genotypes*:** Blood samples were collected from the local breed of cows bred in the province of Basrah (34 samples), as 5 ml were drawn from each animal from the jugular vein using a 10 ml medical syringe after the jugular vein was cleaned and sterilized with 70% ethyl alcohol. Blood samples were placed in tubes containing an anticoagulant (Ethylene Diamine Tetra Acetic Acid - EDTA) and then preserved by freezing at -18°C until DNA extraction steps were performed. The DNA was extracted using the extraction kit (Kit) provided by the Iranian company Genex. The extraction steps were followed according to the instructions of the equipped company. The DNA was detected using an agarose gel at a concentration of 1%. The concentration and purity of the DNA for each sample was measured by a Nano Drop device equipped from Thermo scientific. The genotypes of the DGAT1K232A gene were determined by the method of polymorphisms length restriction fragments polymerase chain reaction (PCR-RFLP). The fragment is located in the exon8 segment of the DGAT1 gene.

Primers for the gene (5'- GCACCATCCTCTTCCTCAAG-3′-forward strand, 5'- GGAAGCGCTTTCGGATG-3′reverse strand) were used to amplify the 411pb segment of the bovine DGAT1 gene containing a lysine/alanine substitution (Exon8). The primers used in the study based on Winter's. et al., (2002) based on the sequence of the DGAT1 gene and on the NCBI GenBank. The PCR reaction was carried out in a volume of 25 µL consisting of Master Mix (12.5) µL, primer F- (1 µL), forward and reverse R- (1 µL), DNA (5) µL, and distilled water (5.5) µL. The PCR reaction was performed using the following program: initial denaturing stage at 94°C for 3 minutes, 35 cycles at 94°C for 45 seconds, annealing stage, extension 62° for 60 seconds, elongation 72° for 60 seconds. The final stage is 72° for 5 minutes. The PCR product was detected using a 2% agarose gel. Random Fragments Length Polymorphism (RFLP) technique was used to determine the genetic polymorphisms of the DGAT1 gene locus and the method was applied to the PCR product according to Ciecierska et al. (2013) with some necessary modifications. The genetic structure of the DGAT1 gene was detected using the Taq1 enzyme, as the TCGA sequence of the front strand is cut between the two TC bases, while the back strand is cut in the AGCT sequence between the two CT bases. The concentrations of the DNA samples were adjusted to 1 ng / microliter. The digestion mixture was prepared for the samples without the restriction enzyme and mixed with a micropipette, then the restriction enzyme was added to the mixture, so that the final volume was 20 microliters. It was placed in a centrifuge for several seconds. The concentrations of the materials involved in the following reaction were DNA7 µL, Taq10.5, Acetylated BSA, 10µg/µl 0.2 µL, RE Buffer 10x2 µL, distilled water 10.3 The samples were incubated in a water bath at a temperature of 65 °C for an hour. One, the resulting alleles were detected using an agarose gel at a concentration of 2%. The results of electrophoreses of samples digested with the restriction enzyme Taq1 showed that there are two alleles: A, K, and three genotypes: AA, AK, and KK.

RESULTS AND DISCUSSION

***Daily, weekly, monthly and first stage lactation milk yield*:** Table 1 shows the average daily, weekly, monthly and the first stage of milk production for the local and Holstein breeds. There are significant differences ($P < 0.05$) in the daily, weekly, monthly and first stage milk production. The genotype AA of local breed was significantly ($P<0.05$) superior to the genotype AK in daily, weekly, monthly and first stage of lactation milk yield (10.750, 75.25, 322,50, 967.50 kg, respectively), those of AK genotype were 5.250, 36.75, 157.50, and 472.50 kg, respectively. Table 1 shows that there were no significant differences between the two genotypes AK and KK of the Holstein breed in the daily, weekly, monthly milk yield and the first stage (18,000, 126.00, 540.00, 1620.00 kg, respectively, for the genotype AK). While those of the KK genotype were 17.250, 120.75, 517.50, 1552.50 kg, respectively. The two genotypes AK and KK of the Holstein breed were significantly ($P<0.05$) superior to the two genotypes AA and AK of the local breed in daily, weekly, monthly and first stage milk production.

These findings contradicted those of Strzakowska *et al.* (2005), who reported that the genotype KK produced somewhat more milk than the genotype AK in their study of the two breeds of white and black Friesian cows. Additionally, these findings conflicted with Pirzad *et al.* (2014) work on the Iranian Holstein breed, which found that genotype AA produced more milk than genotypes AK and KK. In their study on Czech Holstein cows, Kadlecová *et al.* (2014) discovered that the AA genotype produced more milk on average per day than the two genotypes AK and KK.

***Milk Components*:** According to the findings in Table (10) for the percentages of fat and solids-not-fat (SNF), there are significant differences ($P<0.05$) between the two genotypes AA and AK of the DGAT1 gene. In the local breed, the genotype AA exceeded the genotype AK in terms of the percentages of fat and SNF (5.47, and 9.55%), respectively. While the AK genotype's percentages of fat and SNF (3.96 and 6.83%) respectively, decreased significantly ($P< 0.05$).

Table 1. The association between local and Holstein genotypes with daily, weekly, monthly, and first lactation stage milk production

Breed	**Local**		**Holstien**	
Taq1	**AA**	**AK**	**AK**	**KK**
Number	4	30	4	12
Daily milk yield	10.75^{b}±0.96	5.25^{c}±1.63	18.00^{a}±0.89	17.25^{a}±4.53
Weekly milk yield	75.25^{b}±6.70	36.75^{c}±11.43	126.00^{a}±5.79	120.75^{a}±3.16
Monthly milk yield	322.50^{b}±28.72	157.50^{c}±49.00	540.00^{a}±30.37	517.50^{a}±13.56
1^{st} lactation stage milk yield	967.50^{b}±86.16	472.50^{c}±147.00	1620.00^{a}±81.29	1552.50^{a}±40.70

*Numbers with different superscript horizontally differ significantly at p> 0.05

Table 2. Effect of genotypes on milk components

Breed	**Local**		**Holstien**	
Taq	**AA**	**AK**	**AK**	**KK**
Number	4	30	4	12
Fat%	5.47^{a}±0.32	3.96^{b}±0.52	3.25^{bc}±0.06	2.75^{c}±0.33
Protein%	3.53±1.04	2.82±0.81	2.95±0.06	3.09±0.18
Lactose%	3.80±2.12	3.78±0.00	4.24±0.05	4.44±0.21
SNF%	9.55^{a}±2.78	6.83^{b}±2.05	7.75^{b}±0.19	8.10^{a}±0.37

*Numbers with different superscript horizontally differ significantly at p> 0.05

As for the percentage of protein and lactose, there was no significant difference for both genotypes AA and AK (3.53, 3.80 and 2.82, 3.78%), respectively. The findings revealed that there were no changes in the percentage of fat (3.25, 2.75%) between the genotypes AK and KK of the Holstein breed. While the genotype KK substantially (P< 0.05) outperformed the genotype AK in terms of the percentage of solids (8.10% and 7.75% respectively). Regarding the percentages of protein and lactose, there was no discernible variation between the two genotypes AK and KK. For both AK and KK, the percentages of protein and lactose were 2.95, 4.24 and 3.09, 4.44% respectively.

The table also shows that, in terms of fat content, the genotype AA of the indigenous breed was considerably (P< 0.05) superior than the genotypes AK and KK. The percentage of fat of AA genotype in the local breed was (5.47%). However, the fat percentages of the two genotypes of the Holstein breed, AK and KK, were 3.25% and 2.75%, respectively.

Regarding the percentage of SNF, there was not a significant distinction between the genotypes AA in the local breed and KK in the Holstein breed. Furthermore, there is no apparent distinction between the genotypes AK in terms of the proportion of SNF in the two breeds.

Since the percentage of fat indirectly reflects the milk content of SNF, there is a direct correlation between the percentage of fat and each of the percentages of protein and SNF (Patton, 2017). Strzakowska *et al*. (2005) showed that the AK genotype was superior to the KK genotype in the percentage of fat and protein (4.46, 4.16 and 3.55, 3.45%, respectively), in their study on Friesian cows. The present results did not support their findings. However, this result was consistent with what was found by Carvajal *et al*. (2016), where the genotype AA was superior to the genotypes AK and KK in both the percentage of fat and solid non-fat (5.11 and 9.6 %, respectively).

Numerous studies have demonstrated the connection between the combination of SNPs in two or more genes, as well as in the same gene, and milk output. When the genotype AA cows displayed a larger proportion of fat than the other genotypes AK and KK, the results were consistent with those of our study. Al-Niyazi (2016) found that the mixed genotype AK for both strains had a similar proportion of fat and protein to that of the genotypes AA, AG, and GG, which were 3.61, 3.58, 3.47, 3.24, 3.34, and 3.33, respectively, when it was investigated on local and Friesian cows.

Mao *et al*. (2012) in his study on Holstein, the KK genotype was superior in fat and protein percentages compared to the AA and AK genotypes. However, Pirzad *et al*., (2014) in a study on Holstein, where the AA genotype did not outperform the two genotypes AK and KK in the percentage of fat (3.06, 3.2, and 3.3%), respectively. Kadlecová et al. (2014) in his study on Holstein found a lower percentage of fat and protein in the genotype AA compared to the genotypes AK and KK, reaching 3.98, 3.19, 4.03, 3.25, 4.04, 3.29, respectively.

It is noted from Table 2 that the cows bearing the genotype KK were unable to show an ideal ratio of fat and protein, which confirms that there is a negative balance of energy throughout the milk production period for the cows, which is negatively reflected on their health and reproductive condition, or the reason may be attributed to the lack of adaptation of this genotype or insufficient feed materials available to breeders (Al-Niyazi, 2016).

***Conclusion*:** The results showed the presence of three genotypes: AA, AK, and KK. In terms of the quantity of daily, weekly, monthly, and first stage milk production, the findings

also demonstrated that the genotypes AK and KK of the Holstein breed were superior than the two genotypes AA and AK of the local breed. While the genotype AA of the local breed was superior to the genotype AK in terms of daily, weekly, and monthly milk production as well as the first stage, it was also discovered that there is a correlation between the genotypes and the chemical components of milk, as the results showed that the genotype AA was significantly superior by the percentage of fat in the local breed on the AK and KK genotypes of both breeds.

***Conflict of interest*:** The authors declare no conflict of interest.

***Ethical Regulation*:** All procedures of study is conducted under international ethical regulations and based on Univeristy of Basra committee ethical allowance.

Availability of data and material**:** We declare that the submitted manuscript is original work, which has not been published before and is not currently being considered for publication elsewhere.

***Authors participation*:** All authors are similarly participating in this research study for publish in JGIAS ll authors read and approved the final manuscript.

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